

We Claim:

1. A method of sequencing a nucleic acid molecule comprising the steps of:
  - (a) hybridizing two or more sequencing primers to one or a plurality of single strands of the nucleic acid molecule wherein all the primers except for one are reversibly blocked primers;
  - (b) incorporating at least one base onto the nucleic acid molecule by polymerase elongation from an unblocked primer;
  - (c) preventing further elongation of said unblocked primer
  - (d) deblocking one of the reversibly blocked primers into an unblocked primer;
  - (e) repeating steps (b) to (d) until at least one of the reversibly blocked primers are deblocked and used for determining a sequence.
2. The method of claim 1, wherein said step (c) of preventing further elongation comprises
  - (a) completing the elongation from the unblocked primer with polymerase and dNTPs; or
  - (b) terminating the elongation with polymerase in a manganese containing buffer, dNTPs, and at least one ddNTP; or
  - (c) terminating the elongation chemically.
3. The method of claim 1 further comprising the step of removing said polymerase, dNTPs, and ddNTPs before said step (d).
4. The method of claim 1 wherein at least one reversibly blocked primer is blocked by a chemical moiety selected from the group consisting of a PO<sub>4</sub> group, a thio group, and a phosphorothiol group.
5. The method of claim 1 wherein at least one reversibly blocked primer has a 3' mismatched end that can be deblocked by contacting said primer with an exonuclease.
6. The method of claim 1 wherein at least one reversibly blocked primer has one or more noncomplementary bases that forms a loop and does not hybridize to the nucleic acid molecule, wherein said one or more bases is not at the 5' or 3' end of

said reversibly blocked primer, and wherein said reversibly blocked primer comprises a dideoxy nucleotide at its 3' end.

7. The method of claim 6 wherein said at least one reversibly blocked primer is unblocked by endonuclease digestion of said one or more noncomplementary bases forming a nick at said one or more noncomplementary base.
8. The method of claim 7 wherein step (b) comprises polymerase elongation at said nick by a strand-displacing polymerase.
9. The method of claim 1 wherein at least one reversibly blocked primer has a sequence of 5'-NUX-3' wherein N represents an oligonucleotide sequence of any length, U is uracil, and X is a dideoxy-nucleotide.
10. The method of claim 9 wherein said reversibly blocked primer is unblocked by Uracil DNA glycosylase and AP endonuclease to generate an unblocked primer with an extendable 3' end.
11. The method of claim 1 at least one reversibly blocked primer has a sequence of 5'-NYZ-3' wherein N represents an oligonucleotide sequence of any length, Y is a modified nucleotide, and Z represents a single nucleotide base; and wherein said modified nucleotide can be deblocked by formamidopyrimidine (fapy)-DNA glycosylase.
12. The method of claim 11 wherein said modified base is selected from the group consisting of 8-oxoguanine, 8-oxoadenine, fapy-guanine, methyl-fapy-guanine, fapy-adenine, aflatoxin B<sub>1</sub>-fapy-guanine, 5-hydroxy cytosine and 5-hydroxy-uracil.
13. The method of claim 1 wherein the nucleic acid molecule is a genomic DNA, cDNA, or episomal DNA.
14. The method of claim 1 wherein at least one sequencing primer hybridizes to a sense strand of the nucleic acid and at least one sequencing primer hybridizes to an antisense strand of the nucleic acid molecule in step (a).
15. The method of claim 1 wherein the polymerase elongation is between 1 and 250 bases.
16. The method of claim 1 wherein said method is performed in a reaction vessel selected from the group consisting of a test tube, a reaction chamber of a

PicoTiter plate, a reaction chamber of an array, and a microencapsulated reaction chamber of a water-in-oil emulsion.

17. The method of claim 1 wherein the nucleic acid molecule is between 100 to 1000 bp in length.
18. The method of claim 1 wherein at least one said strand of nucleic acid molecule or at least one said primers is attached to a solid support.
19. The method of claim 1 wherein at least one strand of said nucleic acid molecule is linked to a solid support.
20. The method of claim 18, wherein at least one said primer is immobilized on a solid support to form an immobilized primer and said at least one strand is linked to a solid support by hybridization with said immobilized primer.
21. The method of claim 20 wherein the solid support is a spherical mobile solid support.
22. The method of claim 1 wherein at least one primer comprise a detectable label.
23. The method of claim 1 wherein the method of determining a sequence is pyrophosphate sequencing or Sanger sequencing.
24. The method of claim 1 wherein the deblocking step comprises contacting a reversibly blocked primer with an agent to remove a PO<sub>4</sub> group on said reversibly blocked primer.
25. The method of claim 24 wherein said agent is selected from the group consisting of polynucleotide kinase and alkaline phosphatase.
26. The method of claim 1 wherein said polymerase is devoid of 3' to 5' exonuclease activity.
27. The method of claim 1 wherein said method determines a first nucleic acid sequence proximate to one end of said nucleic acid molecule and a second nucleic acid sequence proximate to a second end of said nucleic acid molecule.
28. A method of sequencing a nucleic acid molecule comprising:
  - (a) hybridizing a first unblocked sequencing primer to a first strand of the nucleic acid molecule;
  - (b) hybridizing a second blocked sequencing primer to a second strand of the nucleic acid molecule;

- (c) incorporating at least one base onto said first strand by extending said first unblocked primer with a polymerase;
- (d) preventing further elongation of said unblocked primer;
- (e) deblocking the second sequencing primer; and
- (f) incorporating at least one base onto said second strand by extending said second primer with a polymerase;

wherein steps (a) and (b) are performed in any order or simultaneously.

29. The method of claim 28, wherein said step (d) of preventing further elongation comprises

- (a) completing the elongation from the unblocked primer with polymerase and dNTPs; or
- (b) terminating the elongation with polymerase in a manganese containing buffer, dNTPs, and at least one ddNTP; or
- (c) terminating the elongation chemically..

30. The method of claim 29 further comprising the step of removing said polymerase, dNTPs, and ddNTPs after said preventing step.

31. The method of claim 28 wherein said second primer is blocked by a chemical moiety selected from the group consisting of a PO<sub>4</sub> group, a thio group, and a phosphorothiol group.

32. The method of claim 28 wherein said method determines at least a first nucleic acid sequence proximal to a first end of said nucleic acid molecule and determines a second nucleic acid sequence proximal to a second end of said nucleic acid molecule.

33. A method of determining a molecular haplotype of a DNA sample at multiple loci comprising the steps of:

- (a) hybridizing 2 or more sequencing primers adjacent to a plurality of loci in a DNA sample wherein all the primers except for one are reversibly blocked primers and wherein each locus contains a nucleic acid sequence that determines a haplotype;
- (b) determining a haplotype at one locus by polymerase elongation from an unblocked primer;

- (c) preventing further elongation of said unblocked primer;
- (d) deblocking one of the reversibly blocked primers into an unblocked primer;
- (e) repeating steps (b) to (d) until all the reversibly blocked primers are deblocked and used for determining a molecular haplotype.

34. The method of claim 33, wherein said step (c) of preventing further elongation comprises

- (a) completing the elongation from the unblocked primer with polymerase and dNTPs; or
- (b) terminating the elongation with polymerase in a manganese containing buffer, dNTPs, and at least one ddNTP; or
- (c) terminating the elongation chemically..

35. The method of claim 34 further comprising the step of removing said polymerase, dNTPs, and ddNTPs after said preventing step.

36. A method of sequencing a nucleic acid molecule comprising the steps of:

- (a) hybridizing a sequencing primer to one strand of the nucleic acid molecule;
- (b) incorporating at least one base onto said one strand of the nucleic acid by polymerase elongation from said sequencing primer;
- (c) preventing further elongation of said primer;
- (d) repeating steps (a) to (c) on the same strand of nucleic acid or on a different strand of nucleic acid until a desired amount of sequence is determined.

37. The method of claim 36, wherein said step (c) of preventing further elongation comprises

- (a) completing the elongation from the unblocked primer with polymerase and dNTPs; or
- (b) terminating the elongation with polymerase in a manganese containing buffer, dNTPs, and at least one ddNTP; or
- (c) terminating the elongation chemically..

38. The method of claim 37 further comprising the step of removing said polymerase, dNTPs, and ddNTPs after said preventing step.

39. A method of sequencing a plurality of double stranded nucleic acid molecules comprising the steps of:

- (a) for each of the double stranded molecules, separating two strands of each double stranded nucleic acid and attaching each of the two complementary strands to a single bead, to generate a plurality of beads in a single reactor, each bead with both strands of the nucleic acid molecule attached thereto;
- (b) determining the identity of at least one base of one of the strands;
- (c) determining the identity of at least one base of the complementary strand of the nucleic acid molecule.